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# Proceedings The potential use of synbiotic combinations in cereal-based solid food products- A review

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**Abstract:** To date, the most commonly used probiotics in the potential synbiotic combinations (SC)11in cereal-based solid food (CSF) products belong to the Lactobacillaceae family. On the other side,12*Bacillus coagulans* in pasta, and *Saccharomyces boulardii* in cakes could be promising SC in CSF. Inulin13which is followed by  $\beta$ -glucan is the most commonly direct-used prebiotic source of SC in CSF.14Although there are some promising results regarding the hypocholesterolemic effect of SC in CSF,15there is a need for more comprehensive *in vivo* and *in vitro* studies.16

Keywords: cake; biscuit; pasta; co-encapsulation; wall material; hypolipidemic effect

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## 1. Introduction

Synbiotic is defined as "a mixture comprising live microorganisms and substrate(s) 20 selectively utilized by host microorganisms that confers a health benefit on the host" ac-21 cording to the consensus statement of the International Scientific Association for Probi-22 otics and Prebiotics (ISAPP) [1]. Up to now, although there are many studies regarding 23 potential synbiotic combinations in bread, there are limited studies in the literature based 24 on baked goods (cake, biscuit/cookie/cracker) and other cereal-based solid foods (CSF) 25 such as pasta/noodle, breakfast cereal, and waffle, etc., as shown in Table 1. Until re-26 cently, the most commonly used probiotic bacteria in potential synbiotic combinations 27 (SC) in cereal-based solid food products are Lactobacillus acidophilus, Levilactobacillus 28 brevis, Lacticaseibacillus casei, Limosilactobacillus fermentum, Lactiplantibacillus plantarum, 29 and Lacticaseibacillus rhamnosus, which belong to Lactobacillaceae family (Table 1). 30 Moreover, Saccharomyces boulardii and Bacillus coagulans were generally utilized in syn-31 biotic combinations of cake and pasta formulations, respectively, as seen in Table 1. 32 However, the potential of bacteria from the Bifidobacteriaceae family except Bifidobacte-33 *rium bifidum* has not been adequately evaluated. 34

The most utilized wall materials that have the prebiotic potential for preparing 35 co-encapsulated probiotic bacterial strains to develop their stability and viability 36 throughout producing, storing, and handling processes in cereal-based solid food prod-37 ucts are high-amylose maize starch (Hi-maize), chitosan, and some hydrocolloids such as 38 pectin, k-carrageenan, gum arabic, guar gum, xanthan gum, acacia gum, methylcellulose, 39 and carboxymethylcellulose. Nevertheless, the probiotic inclusion with prebiotic coating 40 materials has not been sufficiently assessed in baked goods, as seen in Table 1. Therefore, 41 this study aims to evaluate the potential synbiotic combinations in cereal-based solid 42 food products, such as cake, biscuits, pasta/noodles, and breakfast cereal summarized in 43 Table 1, and their influence on health. 44

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# 2. The effects of potential synbiotic combinations on some cereal-based solid food products

#### Cake

A dramatic reduction ( $\approx$ 7 log CFU) was observed in the unencapsulated *L. plantarum* 4 after the cake-baking process. The encapsulated *L. plantarum* with pectin and maltodex-5 trin which are combined with calcium-alginate led to a significant increase in probiotic 6 protection capacity and thus viability rate after baking. Although there were no signifi-7 cant differences between the combination of pectin or maltodextrin with calcium-alginate 8 regarding probiotic protection capacity after baking, the highest corresponding values 9 were obtained when using encapsulation wall material composed of calcium-alginate 10 (2%) combined with both pectin (0.5%) and maltodextrin (0.5%) which potentially have a 11 higher thermal resistance. In simulated gastric fluids, no L. plantarum cells were detected 12 in cakes with unencapsulated probiotics and encapsulated of them with calcium-alginate 13 individually. This could be attributed to wall materials having prebiotic potential which 14 assists in restricting the porous structure of the microbial composition resulting in 15 strengthening the gel network and thus restraining acid diffusion into the microbial im-16 pact of probiotic life. The probiotic viability was also developed by alginate-based mi-17 crocapsules with pectin and maltodextrin in both simulated gastric- and intestinal-fluids 18 [2]. 19

No viable unencapsulated (S. boulardii, L. acidophilus, and B. bifidum) probiotics were 20 detected irrespectively of probiotic strain in cakes after the baking process. However, 21 double-layered microcapsules, composed of the gum arabic and  $\beta$ -glucan as the inner 22 layer generated by spray-drying, and hydrogenated palm oil as an outer layer generated 23 by spray chilling, enhanced the viability of S. boulardii and L. acidophilus account for 24 nearly 3 log CFU/g after baking. It was explained by the combination of hydrophilic and 25 hydrophobic materials of the inner and outer layers, respectively. It was attributed to re-26 stricting conventional heat transfer by limiting the movement of water. However, no vi-27 able cells of single- or double-layered microencapsulated B. bifidum were defined after 28 cake baking, which was interrelated with lower heat tolerance in comparison to other 29 probiotics [3]. 30

In another study, the *S. boulardii* was inoculated into rice cake made from black glu-31 tinous rice, which has high antioxidant capacity, and prebiotic potential, after starter ad-32 dition to provide synbiotic characteristics. In the simulated gastrointestinal system, a 33 higher survival rate accounting for nearly 97% of probiotic yeast was achieved in fer-34 mented rice cake inoculated with 103 S. boulardii when compared to the control cake con-35 sisting of only rice cake starter without probiotic yeast. This was referred to as limiting or 36 retardation of the influx of acidic fluids into the cells of probiotic yeast, thus preserving 37 them throughout their gastrointestinal tract and also against bile attacks [4]. 38

#### Biscuit

The initial viable probiotic counts were nearly 10 log CFU/g in cream biscuits in-40 cluding encapsulated three probiotic strains (mixture of L. acidophilus, L. rhamnosus, B. bi-41 fidum) with different wall materials (guar gum-inulin-dextrose mixtures or xanthan 42 gum-maltodextrin-sucrose). Therefore, although the number of encapsulated probiotics 43 was decreased by 2 log cycles after 8 storage, it still met the recommended probiotic level 44  $(10^{6}-10^{8} \text{ CFU/g})$ . However, better probiotic viability was obtained in the cookies includ-45 ing encapsulated probiotics based on the mixture of guar gum-inulin than xanthan 46 gum-maltodextrin. The scores of major sensorial properties such as taste and overall ac-47 ceptability were higher in biscuits including probiotics encapsulated with the wall mate-48 rial mixture composed of guar gum-inulin than xanthan gum-maltodextrin. This was at-49 tributed to the aftertaste of xanthan gum. However, the encapsulated probiotic-added 50 biscuits remained acceptable for up to 8 weeks of storage, irrespective of the composition 51 of wall material mixtures [5]. 52

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In another study, the sugar replacement of gluten free-cookies based on corn flour 1 and buckwheat flour was conducted with inulin (naturally-soluble dietary fiber) and jocoque in cookie weight for adding coated *L. brevis*. The color *L* values were reduced because of coating opacity when biofilm formed. The firmness values were also decreased 4 in sugar-replaced cookies coated with a probiotic-forming film, and it was explained by 5 providing moisture into the cookie structure by coating related to moisture distribution 6 [6].

#### Pasta

The number of Bacillus coagulans in uncooked probiotic- and barley flour-added 9 pasta samples was nearly 7 log CFU/g which is consistent with qPCR data but decreased 10 with cooking depending on cooking time, as expected. In this regard, consuming an av-11 erage amount of 100 g of pasta in a meal could be accepted as sufficient to demonstrate 12 beneficial effects on gut microbiota. However, the satisfactory cooking quality of pasta 13 which included barley flour and *B. coagulans* was obtained in 7 min than 5 min cooking 14 time regarding higher weight and firmness values. According to a nutritional point of 15 view, there were no significant differences between glycemic index values of probi-16 otic-enriched pasta including barley flour and control pasta, and medium glycemic in-17 dexes were observed [7]. 18

The viable L. plantarum cells encapsulated with fructooligosaccharides and dena-19 tured whey protein isolate accounted for 93.63% before cooking, and 62.42% of encap-20 sulated cell viability was retained in raw noodles after cooking. This was dedicated to the 21 initial moisture content of raw noodles with encapsulated probiotics which preserve the 22 cell membrane from osmotic shock and thermal damage throughout rehydration. Nev-23 ertheless, the cell viability was 80.29%, and 64.74% in dried noodles at low and high 24 temperatures, respectively. The decrease in probiotic viability, because drying was ded-25 icated to heat stress throughout dehydration, resulted in damage to the cell wall and 26 membrane. Moreover, the encapsulated cell viability was lost in dried noodles after 27 cooking which was referred to as thermal inactivation occurred throughout the rehydra-28 tion of dried cells because of leakage of fundamental cellular compounds. A shorter 29 cooking time was needed for raw pasta including encapsulated L. plantarum when com-30 pared to control pasta with no probiotic. This was explained by decreasing gluten levels 31 with the incorporation of probiotic microcapsules led to more quick starch gelatinization. 32 The solid loss of both raw and dried pasta at low and high temperatures including en-33 capsulated probiotics was higher than control pasta. It was referred to discontinuous 34 protein matrix due to the distribution of probiotic microcapsules into the gluten network. 35 No significant differences were determined in sensorial properties such as sweetness, 36 firmness, and chewiness between probiotics including raw noodles with respect to con-37 trol [8]. 38

#### Other cereal-based solid foods

The S. boulardii viability was highest with acacia gum due to thermal protection and 40 preventing oxidative damage, but the lowest with carboxymethylcellulose, and methyl-41 cellulose compared to other coating agents such as modified starch and maltodextrin, 42 when coated breakfast cereals exposed to pre-heated-milk at 70°C and 80°C. The lowest 43 viability of coated the probiotics on breakfast cereals with cellulose-derivative hydro-44 colloids was attributed to their higher viscosity at low concentrations resulting in the 45 formation of a permeable surface after drying. An increase in the coating concentration of 46 acacia gum as a coating material from 2.5% to 10% led to an increase in viability and 47 thermal protection of *S. boulardii* when exposed to pre-heated milk at different tempera-48tures (50, 60, 70, and 80°C). This was explained by layer formation which makes a barrier 49 for heat penetration around S. boulardii. The coating with acacia gum showed approxi-50 mately 12% times higher viability compared to coating S. boulardii without acacia gum in 51

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simulated intestinal juice. Therefore, it was stated that the coating not only preserved *S*. 1 *boulardii* but also enhanced its viability [9].

The probiotic culture inoculated yoghurt at 4 different concentrations (0.5, 1.5, 3, and 3 4.5%) enriched with inulin and lactulose (disaccharide derivative of lactose) as a prebiotic 4 source at 3% of each was used in tarhana production. The number of probiotics was in-5 creased with concentrations of probiotic culture, and the highest values were acquired in 6 2. days of fermentation irrespective of the probiotic strain. On the 2nd day of fermenta-7 tion, the highest microbial count generally belonged to L. acidophilus followed by S. 8 thermophilus, and B. bifidum, respectively in tarhana dough. Although the microbial 9 counts of each probiotic strain were increased with the rise in probiotic concentration in 10 dried tarhana, the drying process negatively influenced the microbial counts compared 11 to tarhana dough. However, the sensorial attributes such as flavor and texture were still 12 above 4.5 on a 5-point hedonic scale [10]. 13

Table 1. The potential use of synbiotic combinations in some cereal-based solid food products.

	Product	Probiotic source(s)	Prebiotic or potential prebiotic source(s)	References
CAKE	Cupcake	Lactiplantibacillus plantarum	Pectin <sup>b</sup> , maltodextrin <sup>b</sup>	[2]
	Cupcake	Lactiplantibacillus plantarum	к-carrageenan <sup>ь</sup>	[11]
	Cream-filled cake	Lacticaseibacillus casei	High-amylose resistant starch <sup>b</sup>	[12]
	Cake	Saccharomyces boulardii, Lactobacillus acidophilus, Bifidobacterium bifidum	Gum arabic <sup>b</sup> , $\beta$ -cyclodextrin <sup>b</sup>	[3]
	Fermented rice cake (Khao-Maak)	Saccharomyces boulardii	Germinated black glutinous riceª	[4]
	Muffin	Lactiplantibacillus plantarum	Stevia rebaudiana <sup>a</sup>	[13]
	Gluten-free cake mix	Bacillus coagulans	Inulin <sup>a</sup> , resistant starch <sup>a, x, z</sup> , maltodextrin <sup>a, x</sup>	[14]
BISCUIT/ COOKIE/ CRACKER	Cracker	Lacticaseibacillus casei	Inulin <sup>b</sup> , whey <sup>b</sup>	[15]
	Biscuit cream	Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, Bifidobacterium bifidum	Inulin <sup>ь</sup> , guar gum <sup>ь</sup> , xanthan gum <sup>ь</sup> , maltodextrin <sup>ь</sup>	[5]
	Gluten-free cookie	Levilactobacillus brevis	Inulin <sup>a, x</sup>	[6]
	Gluten-free biscuit	Lactobacillus acidophilus	Inulin <sup>b</sup> , fructooligosaccharide <sup>b</sup>	[16]
OTHERS PASTA/ NOODLE	Pasta	Bacillus coagulans	Barley flour <sup>a</sup>	[7]
	Pasta	Lactiplantibacillus plantarum, Lactobacillus acidophilus, Limosilactobacillus fermentum	β-glucanª	[17]
	Noodle	Lactiplantibacillus plantarum	Fructooligosaccharide <sup>b</sup>	[8]
	Whole-grain pasta	Bacillus coagulans	β-glucanª	[18]
	Breakfast cereal	Saccharomyces boulardii	Acacia gum <sup>b</sup> , methylcellulose <sup>b</sup> , carboxymethylcellulose <sup>b</sup> , modified starch <sup>b</sup> , maltodextrin <sup>b</sup>	[9]
	Wafflefilling	Lactobacillus acidophilu, Bifidobacterium bifidum	Inulin <sup>a, x</sup> , pectin <sup>b</sup> , lactulose <sup>a,y</sup>	[19]
	Traditional fermented food (Tarhana) <sup>t</sup>	Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacterium bifidum	Inulin <sup>a</sup> , lactose <sup>a</sup>	[10]

a: direct usage, b: coating, x: used as a fat replacer, y: used as a sugar replacer, t: prebiotics were used in yoghurt for tarhana production, z: type of resistant starch is not defined.

# 3. The effects of potential synbiotic combinations in some cereal-based solid food products on health

The feeding of experimental rats with synbiotic biscuits (5g or 10 g in 10 mL 19 aquadest) including *L. acidophilus*, inulin, and fructooligosaccharide led to a significant 20 decrease in total blood cholesterol levels. Moreover, an increase in HDL levels was ob- 21

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served and it was explained by the fermentation ability of probiotics which cause a de-1 crease in pH values and thus an increase in H+ ions in the intestine which led to an in-2 crease in water links with lipids through lipoprotein. In contrast, a decrease in LDL levels 3 was recorded and referred to decrease in triglyceride synthesis due to the inhibition ef-4 fect of inulin on lipogenic enzymes in the liver, and also the fermentation of inulin by 5 probiotics which induce the generating of the short-chain fatty acids such as propionic 6 acid [16]. According to the results of a single-blind, parallel, randomized, placebo study, 1 7 serving/day consumption of potential synbiotic whole-grain pasta composed of B. coag-8 *ulans* and  $\beta$ -glucans for 12 weeks by healthy overweight or obese volunteers (*n*=41), the 9 plasma LDL/HDL cholesterol ratio was decreased [18]. In another study, the consump-10 tion of 200g/day dried tarhana, which is prepared from yoghurt containing inulin (3%) 11 and lactulose (3%) fermented by 4.5% probiotic culture, for 45 days led to a significant 12 decrease in total plasma cholesterol and triglycerides in hyperlipidemic volunteers 13 (n=15). Therefore, it was declared that the potential synbiotic tarhana has a significant 14 hypocholesterolemic effect regarding the influence on the plasma lipid profile of human 15 subjects. This was mainly referred to as behaving as a soluble fiber and could not hy-16 drolyzation by the human digestive system and thus shows a hypolipidemic effect. 17 Moreover, the lowering cholesterol effect was also attributed to  $\beta$ -glucan from wheat 18 flour, and other tarhana ingredients such as onion, green pepper, and tomato due to its 19 lycopene content [10]. 20

### 4. Conclusion

The Lactobacillaceae family is the most commonly preferred probiotic in the poten-22 tial synbiotic combinations in cereal-based solid foods (cake, biscuit/cookie, pasta/noodle, 23 etc.). In other respects, the promising probiotics such as Saccharomyces boulardii and Ba-24 cillus coagulans were evaluated in cakes and pasta, respectively were evaluated with 25 prebiotics/potential prebiotics which could have synbiotic potential. In this regard, the 26 major direct-used prebiotic sources were inulin and  $\beta$ -glucan regarding potential synbi-27 otic combinations in cereal-based solid foods. Consequently, future in vivo and in vitro 28 studies should be centered around the survivability of more probiotic microorganisms, 29 especially the lack of the Bifidobacteriaceae family, optimization of the encapsulation 30 process, with different prebiotic sources at different levels utilized in particularly glu-31 ten-free cereal-based solid food products. Moreover, not only the viability of probiotics 32 with prebiotics but also the nutritional, technological, and sensorial properties of cere-33 al-based solid food products should also be evaluated regarding their synbiotic potential. 34 The potential synbiotic combinations in cereal-based liquid food products such as juic-35 es/beverages should be addressed in other studies. From the human health perspective, 36 there is a requirement for more comprehensive in vivo and in vitro studies regarding the 37 hypocholesterolemic effect of potential symbiotic combinations in cereal-based solid 38 foods. 39

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